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Selection of a sublibrary enriched for a chromosome from total human bacterial artificial chromosome library using DNA from flow sorted chromosomes as hybridization probes

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The division of total genomic libraries into chromosome-specific sublibraries is critical for efficient analysis and mapping of the genome. A new colony hybridization-based approach to rapidly identify chromosome-specific clones from a human genomic bacterial artificial chromosome (BAC) library (1,2) is described. The most ideal hybridization probe for identifying all of the chromosome-specific clones from a genomic library in a single hybridization would be the chromosome itself. Using flow sorted chromosome 22 DNA as a probe, which was obtained as previously described (3,4), we screened a 4× human genomic BAC library gridded onto 42 hybridization filters at 5 × 5 density using a Biomek1,000 gridding tool (2). 100 ng of sorted DNA was labeled with [α-^{32}P]dCTP and [α-^{32}P]dATP by random priming and hybridized to the filters under suppressive condition (2). Chromosomes 21 and 22 are similar in size, and they both are acrocentric chromosomes carrying ribosomal repeats. Therefore we also hybridized the library filters with sorted chromosome 21 DNA probe as a control. Comparison of hybridization results from both chromosomal DNA allowed us to identify many of the non-chromosome-specific false positives that arose from cross hybridization between highly repetitive elements present in different chromosomes. The hybridization images were digitized via PhosphorImager (Molecular Dynamics) and processed using a computer program (5) that extracts the coordinate and intensity information for each colony on the filters. Due to the highly complex nature of the chromosomal probes, the hybridization suffered from background noise as well as low hybridization signal intensity. Quantitation and analysis of numerous weak signals with varying intensities necessitated the use of a detection method more sensitive than exposing to X-ray films and a computer based approach to read the hybridization images. Colonies belonging approximately to the top 5% intensity group from each filter were selected from each chromosomal hybridization. Most of the clones with strong signals in both chromosomal hybridizations turned out to contain ribosomal and other repeats (Fig. 1). After excluding these common hits, ~3300 and 3000 colonies with unique strong signals were picked for chromosome 22 and 21 sublibraries, respectively (6). Figure 2 shows the distribution of some previously known chromosome 22- and 21-specific clones along the gradient of hybridization signal intensities. The majority of chromosome 22- and 21-specific clones were present in the top 5% group. We randomly picked 82 clones from the chromosome 22 sublibrary and localized them on

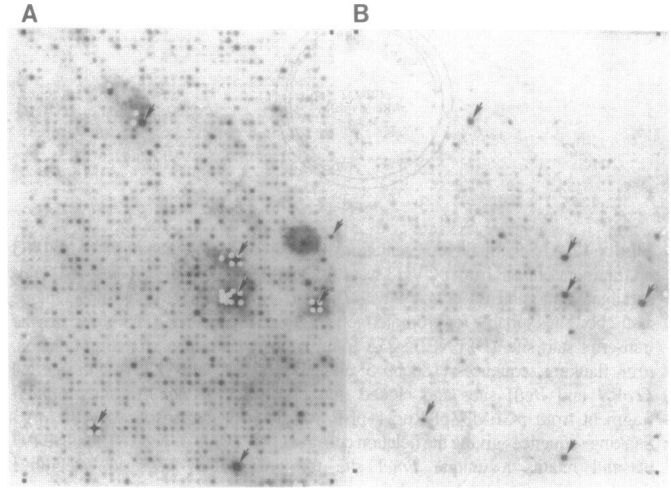

Figure 1. Examples of the digitized images from the hybridization of sorted chromosome 22 DNA (**A**) and chromosome 21 DNA (**B**) to identical BAC colony filters gridded at 5 × 5 format. Arrows indicate clones that hybridize strongly to both chromosomal probes. Most of these clones were shown to be ribosomal or centromeric repeats (not shown). Corners are indicated by solid rectangles. A filter gridded in 5 × 5 density of 96-well titer plate format contains 2304 colonies (because the last position was left empty to help localize the blocks more easily), and represents 1/42 of the total library. The total library was therefore gridded on 42 separate filters. In practice, some of the filters generated uninterpretable images due to unknown technical errors, and only 36 and 32 out of 42 filters for chromosomes 22 and 21, respectively, were processed.

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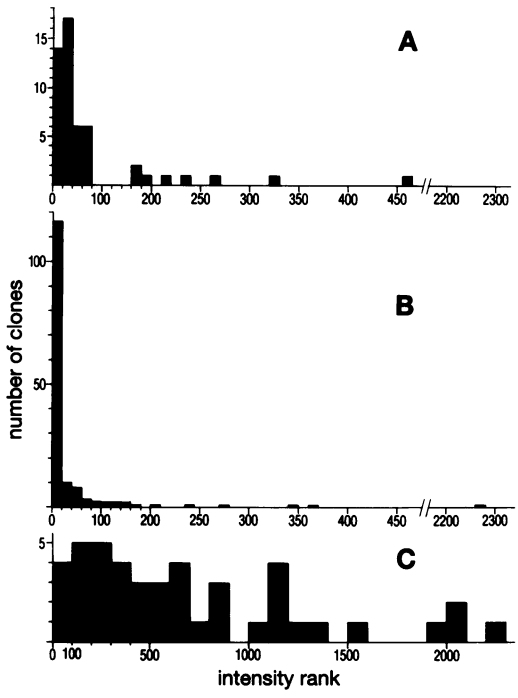

Figure 2. Distribution of known BAC clones as a function of relative hybridization signal intensities. For each filter, clones were ranked in the order of signal intensities (1–2304; 1 being the colony with the strongest signal in the filter). This way, every clone in the library is assigned with a rank, and each rank has 42 corresponding clones. Fifty-nine chromosome 22-specific clones (**A**), 171 ribosomal clones (**B**) and 45 clones that were mapped to other chromosomes (**C**) were sorted according to their ranks.

the chromosomes by FISH analysis (7,8), and found that 33 clones or 39.5% of them were on chromosome 22 (not shown). This represents >20-fold enrichment of the chromosome-specific clones from the total genomic library, and indicates that the sublibrary would contain ~1200 chromosome 22-specific clones. Because we estimate that 1500–2000 chromosome 22-specific BAC clones would exist in the original 4× library, we conclude that a majority of chromosome 22-specific clones were selected in this approach.

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